A Simplified Method for the Estimation of Diastase In Honey

ABNER I. SCHEPARTZ
and
MARY H. SUBERS

Eastern Regional Research Laboratory[1]/
Philadelphia, Pa.

IN RECENT YEARS the enzyme known as diastase has become very important to exporters of American honey, since its presence in honey is used as a criterion of acceptability on the European market. Shipments of honey are sometimes refused or downgraded and sold at reduced prices, because they do not meet the minimum requirement for diastase.

The analytical determination of diastase is performed in only a few laboratories in this country. In some cases it has been necessary for those interested in diastase to send samples a long distance to one of these laboratories. The method of analysis for diastase generally used by these laboratories is that developed by Schade **et al**, (1958). It has been modified by White (1959) to correspond with the European methods. The procedure consists of allowing the diastase in a honey sample to act upon a starch solution under standardized conditions. The starch is broken down into smaller and smaller units. The progress of this breakdown is followed by a color test with iodine. Initially, the intact starch gives a deep blue color with iodine. As the starch is broken down the color test passes through a series from blue to purple to red to brown and finally yellow. The time required to reach a particular, accurately determined color is an inverse measure of the amount of diastase present in the honey. It is expressed as a "diastase number". This method requires the use of precision analytical laboratory equipment and apparatus. Without proper facilities, it cannot be used. It has, therefore, become imperative that a simplified test be made available to those with only limited laboratory facilities whereby an approximation of the diastase number could be determined. An estimate of this type would be of assistance in selecting a particular lot of honey for export.

The proposed procedure is a simplification of the modified Schade method. The visual observance of a brown color in the starch-iodine test is taken as the end-point. The rapidity with which a honey sample causes this brown color to appear is a measure of its diastase content.

The new method was calibrated against the modified Schade by analysis of a series of 27 honey samples of different floral types chosen to give a variety of diastase numbers. In this way end-point times in the new method could be related to the true diastase numbers as determined by the modified Schade method. Since the simplified procedure is designed to be run at room temperature without thermostatic control, these analyses were performed at room temperature (80°F) and at 90°F

[1]/Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

(to approximate summertime conditions). The calibrations were expressed as ranges and set up in tables as shown below (Tables 1 and 2). Thus, for any given end-point, in terms of the time required to reach a brown color, one may judge the diastase number to be in a corresponding range. In some cases the ranges overlap. At 90°F the end-point is generally reached five minutes earlier than at 80°F, but since this is not strictly true throughout the series, both tables are given.

Since the current practice in Europe is to reject or down-grade any honey with a diastase number of eight or less (Duisberg and Gebelein (1958); White **et al.**, (1964)), a lower limit before export of about 10 might be fairly realistic. Thus, we see that a honey reaching the end-point in 25 minutes at 80°F or 20 minutes at 90°F would be at the lower limit.

Principal of the Method

The method is based on the more precise technique of Shade **et al.**, (1958), as modified by White (1959), utilizing the disappearance of the starch-iodine blue color with increasing diastatic activity. It is designed for use where only a minimum of equipment is available. The end-point is determined visually and can be related to the actual diastase number as determined by the modified Schade method.

Apparatus

(a) **Balance** - An ordinary laboratory balance capable of weighing to the nearest 0.1 gram. This may be a single or double pan balance such as an Ohaus Harvard Trip[2]/ type, which is available from any chemical laboratory supply house at a cost of about $20-30.

(b) **Test Tubes** - Any small tubes such as 3" x ¼" in size.

(c) **Test Tube Rack** - Small rack for above tubes.

(d) **Medicine Droppers** - Ordinary medicine droppers with 3" glass tubes and rubber bulbs.

(e) **Glassware for Dilutions** - Graduated cylinders may be used where volumetric flasks are not available.

[2]/Mention of trade or company names does not imply endorsement by the Department over others of a similar nature not named.

Reagents

(a) **Phosphate-Citrate Buffer** - Weigh out 2.0 g citric acid monohydrate ($H_3C_6H_5O_7 \cdot H_2O$) (available from J. T. Baker Chemical Company) and 5.7 g sodium phosphate, dibasic heptahydrate ($Na_2HPO_4 \cdot 7H_2O$) (J. T. Baker Chemical Company). Dissolve both together in 100 ml H_2O[3]/. This gives a buffer of pH 5.1 which is 0.2M in regard to phosphate.

(b) **1% Starch Solution** - Weigh out 1.0 g starch (soluble-potato powder for iodometry, J. T. Baker, Reagent Grade, No. 4006) and dissolve in 100 ml water by boiling over a low flame for three minutes.

(c) **N/100 Iodine Solution** - Dilute 1 part of N/10 iodine solution (Fisher Scientific Company, Certified Reagent, No. So-1-86) with 9 parts of water as needed daily in small volumes, keeping the N/10 as a stock.

Procedure

Dissolve 5.0 g of honey in 25 ml of the buffer. Into each of a series of 7 tubes in a rack place 10 drops of this honey solution and 10 drops of water. An eighth tube is prepared as a blank with 10 drops of buffer and 10 drops of water. At time zero add 1 drop of 1% starch to each tube, mix thoroughly and allow to stand at room tempera-

Table 1

Approximate Diastase Numbers at 80°F

Time (min.)	Approx. Diastase No.
5	32 and higher
10	24 - 31
15	18 - 23
20	13 - 18
25	10 - 15
30	9 - 13
35 and higher	Below 10

Table 2

Approximate Diastase Numbers at 90°F

Time (min.)	Approx. Diastase No.
5	30 and higher
10	20 - 29
15	14 - 20
20	10 - 15
25	8 - 12
30 and higher	Below 10

[3]/Distilled water should be used wherever possible.

ture (about 80°F). At any time during the first 5 minutes add 1 drop of N/100 iodine solution to the blank tube, mix and allow to stand. The blue color obtained should remain constant during the entire procedure as a check on the reagents. At time 5 minutes add 1 drop of N/100 iodine solution to the first honey tube, mix and note the color immediately. The appearance of a definite brown color is the end-point. If this color is not obtained in the first tube, at time 10 minutes add 1 drop of the iodine to the second tube, at 15 minutes to the third tube and so on at 5 minute intervals until the end-point is reached. Note the time for the tube showing the brown color and refer to the proper table for the corresponding diastase number.

REFERENCES

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